REMARKS

I. Disposition of Claims

Claims 4, 5, and 9-12 are currently pending. Claims 4, 5 and 9-12 are currently amended. Support for the amended claim can be found throughout the specification, for example in the original claims, and on page 8, lines 6 to 9.

II. Written Description

The Examiner has rejected claims 4-5, and 9-12 under 35 USC 112, first paragraph, as failing to comply with the written description requirements.

The Examiner asserts the claims, by reciting a peptide belonging to the thioredoxin (TRX) superfamily, do not provide sufficient recitation of distinguishing identifying characteristics and structure/function relationship.

As amended, the claims now recite a polypeptide comprising "the sequence selected from the group consisting of -Cys-Gly-Pro-Cys-, -Cys-Pro-Tyr-Cys-, -Cys-Pro-His-Cys- and -Cys-Pro-Pro-Cys-, and having an activity of alleviating the symptoms of inflammatory bowel disease". The amended claims provide both structural and functional limitations to the polypeptide and therefore the claims should be considered in compliance with the written description requirement.

III Enablement

The Examiner rejects Claims 4-5 and 9-12 Under 35 USC 112, first paragraph for lack of enablement. (Section 2b on page 4 in the Office Action). Under MPEP 2164, the test for enablement is whether one skilled in the art could make or use the subject matter defined by the claims without undue experimentation.

(i) The Examiner asserts that the specification discloses a method for alleviating colitis symptoms in dextran sulfate sodium (DSS)-induced colitis in TRX-Tg mice, but not the invention defined in the original Claims related to a method for treating patients having the inflammatory bowel disease by administering "all/any" members of the thioredoxin superfamily.

As described above, the claims are now limited to a polypeptide comprising a specific structure; the claims now recite "the sequence selected from the group consisting of -Cys-Gly-Pro-Cys-, -Cys-Pro-Tyr-Cys-. -Cys-Pro-His-Cys- and -Cys-Pro-Pro-Cys-, and having an activity of alleviating the symptoms of inflammatory bowel disease". In the Example, -Cys-Gly-Pro-Cys

(recombinant human thioredoxin) is used, and the effect thereof is confirmed (page 14, line 15 to page 17, line 5 of the specification).

Those skilled in the art will easily understand that the use of a polypeptide containing the structure in the active site in which activity is the same as that of thiordoxin, i.e.., -Cys-Pro-Tyr-Cys-, -Cys-Pro-His-Cys- and -Cys-Pro-Pro-Cys, will achieve similar effects. Therefore, the rejection should be withdrawn.

(ii) The Examiner also asserts that the specification does not describe an example of the treatment of ISD by administering a peptide belonging to the thioredoxin superfamily.

As shown in Example 2, after dextran sulfate sodium (DSS) is administered to C57Bl/6 mice to prepare colitis model animals, recombinant human thioredoxin is administered. The use of human theoredoxin is, of course, the use of a peptide covered by the scope of the amended claims. Further, as shown in Figures 7, 8-A and 8-8 in the specification, colitis symptoms are significantly ameliorated by human thioredoxin peptide administration. Thus, the specification details an example representative of the genus of the scope of the claim.

In view of the foregoing, those skilled in the art could practice the invention referring to the description of the specification.

Wands factor analysis

Under MPEP 2164.01(a), the Wands factors are to be considered in determining whether any necessary experimentation is undue. The presence or absence of working examples is but one of the factors to be considered.

i) First, there is considerable direction and guidance in the specification with respect to how to make and use the subject matter defined in the claims.

- **The specification describes the measures required to make and use the claimed embodiment at page 7, line 25 to page 12, line 6:**

The family exhibiting thioredoxin activity contains the sequence -Cys-X-Y-Cys- in the active site (wherein X and Y are the same or different amino acids selected from 20 kinds of natural amino acids and is called a thioredoxin superfamily (hereinafter sometimes referred to as the "TRX family").

Examples of polypeptides of the TRX family include those having the sequences: -Cys-Gly-Pro-Cys-, -Cys-Pro-Tyr-Cys-, -Cys-Pro-His-Cys-, or -Cys-Pro-Pro-Cys- in the active site. Among these, preferable are those having the sequence -Cys-Gly-Pro-Cys- in the active site.

Specifically, examples of polypeptides belonging to the TRX family include thioredoxins derived from animals including humans (ADF derived from animals including humans), thioredoxins derived from bacteria such as E. coli, thioredoxins derived from yeasts, and other thioredoxins; polypeptides having human ADF activity (human ADFP); glutaredoxins derived from humans, *E. coli*, etc.; and the like.

Preferable polypeptides belonging to the TRX family are thioredoxins, and especially preferable are human and yeast thioredoxins. Yeast thioredoxins may be isolated from yeast or may be in the form of yeast containing significant amounts of thioredoxin.

Such polypeptides of the TRX family can be used alone or in combination in the therapeutic agent for treating an inflammatory bowel disease of the present invention.

Polypeptides belonging to the TRX family can be obtained from bacteria (e.g., *E. coli*), yeasts, plants, and animals, especially mammals (humans, cows, horses, dogs, cats, monkeys, guinea pigs, rats, mice, rabbits, etc.). Polypeptides belonging to the TRX family can be obtained according to methods for purifying natural products or according to genetic engineering using yeast, *E. coli*, etc.. Polypeptides, insofar as exhibiting TRX activity, may be derivatives in which one or a plurality of, and preferably one or a few, amino acids are substituted, added, or deleted.

Polypeptides including thioredoxin can be either in the oxidized form or in the reduced form, but preferably in the reduced form.

Examples of intestinal diseases to be treated are inflammatory bowel diseases, for example, ulcerative colitis; Crohn's disease; infectious colitis caused by bacteria, parasites, viruses, fungi, etc.; drug-induced colitis caused by medicines, chemical compounds, etc.; irradiation colitis; ischemic colitis; obstructive colitis; solitary rectal ulcers; regional colitis; hemorrhagic colitis; etc.

Administrative routes for the therapeutic agent of the present invention for treating an inflammatory bowel disease can be either oral or parenteral, and suitably selected by clinicians. The active ingredient, thioredoxin, can be administered singly or in combination with conventional carriers.

When administered orally, the prophylactic and therapeutic agent of the invention may be in the form of tablets, coated tablets, powders, granules, capsules, pills, or like solid formulations; solutions, suspensions, emulsions, syrups, or like liquid formulations; aerosols, atomizers, nebulizers, or like inhalants; liposome-encapsulated formulations; etc.

When administered parenterally, the prophylactic and therapeutic agent of the invention may be in the injectable form (e.g., solutions, suspensions, etc.) intended for intravenous, subcutaneous, endodermic, intramuscular, intraperitoneal, and like injections; and may be in other forms such as solutions, suspensions, emulsions, drops, suppositories, ointments, etc.

When the prophylactic and therapeutic agent of the invention is in the form of a solution, it may be frozen and stored, or freeze-dried to remove its water content and stored. Freeze-dried formulations may be used by redissolving them in distilled water for injection or like liquid media.

Examples of pharmaceutically acceptable carriers for use in the prophylactic and therapeutic agent of the invention include diluents and excipients that are usually used according to the form of pharmaceutical preparation, such as binders, disintegrants, surfactants, absorption enhancers, humectants, adsorbents, lubricants, fillers, extenders, moisturizers, antiseptic agents, stabilizers, emulsifiers, solubilizers, salts for adjusting osmotic pressure, buffers, etc. These carriers are suitably selected according to the unit dosage form of the prophylactic and therapeutic agent.

Furthermore, colorants, preservatives, aroma chemicals, flavorings, sweeteners, and the like, as well as other pharmaceuticals may be used in the prophylactic and therapeutic agent of the invention if necessary.

The amount of polypeptide of the TRX family effective for treating an inflammatory bowel disease can be readily selected by a person skilled in the art in view of conventional techniques. For example, it is generally about 0.1 to about 100 mg, preferably about 0.1 to about 10 mg, and more preferably about 1 to about 10 mg, per kg body weight per day, which may be attained in 1-3 doses. It is preferable to select the amount according to the form of therapeutic agent, gender of the patient, age, degree of the disease, etc.

The prophylactic and therapeutic agent of the present invention can be used in steroid hormone therapy, which is currently the primary therapeutic method for inflammatory bowel diseases, thereby contributing to the reduction or withdrawal of the use of steroid hormones.

- **The specification provides the prophetic example of Example 2:**

Example 2. Investigation of Therapeutic Effect of Recombinant Human Thioredoxin on DSS-Induced Colitis Model Mice

C57BL/6 mice were given 3% (w/v) DSS in their drinking water for 5 days, and reared 5 more days with pure water till Day 10. These mice were weighed daily. From immediately before the beginning of DSS administration (Day 0), 150 μl of phosphate buffered saline (PBS) was intraperitoneally administered to the Control Group (n = 10) every day, and 5 mg/kg of human recombinant thioredoxin dissolved in 150 μl PBS was intraperitoneally administered to the Treatment Group mice (n = 10) every day. At Day 10 of the therapy, the mice were sacrificed by ether anesthesia, and the whole colons were removed to examine colitis.

The weight reduction over Day 5 to Day 10 (DSS+5) after administration of DSS was significantly smaller in the Treatment Group than in the Control Group (Figure 5). When measured, the average whole colon length of the Treatment Group was notably longer than that of the Control Group (Figure 6).

A histological investigation by HE staining revealed that the tissue damage observed in the Control Group was significantly rectified in the Treatment Group (Figure 7). Data points generated by the Histological scoring system show significant rectification in the Treatment Group with respect to all categories Inflammation Severity, Inflammation Extent, Crypt Damage and Total Colitis (Figure 8-A and B).

As demonstrated by the Examples, (1) the investigation using TRX-Tg mice showed the importance of TRX in colitis inhibition, and (2) therapeutical administration of TRX alone can rectify pathological conditions of colitis. These facts suggest the effectiveness of TRX as a therapeutic agent for treating inflammatory bowel diseases such as ulcerative colitis and the like.

ii) Second, there was a high level of skill in the art at the time the application was filed. The level of skill in the molecular biology art was that of a postdoctoral fellow working in the laboratory. *Amgen Inc. v. Hoechst Marion Roussel Inc.*, 57 USPQ2d 1449, 1518 (D. Mass. 2001). Thus, the level of skill in the art was high.

iii) Third, all of the methods needed to practice the invention were well known. As of the 5 April 1994 priority date, for guidance regarding such conditions, see, for example, Sambrook, et al., 1989, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York; and Ausubel et al., 1989, Current Protocols in Molecular Biology, Green Publishing Associates, Inc., and Wiley & Sons, Inc., New York.

iv) Per MPEP 2164.01(a), the In re Wands Court held that the specification was enabling with respect to the claims at issue and found that "there was considerable

direction and guidance" in the specification; there was "a high level of skill in the art at the time the application was filed;" and "all of the methods needed to practice the invention were well known." Similarly, here, as indicated above, there was considerable direction and guidance in the specification; there was a high level of skill in the art at the time the application was filed; and all of the methods needed to practice the invention were well known. Thus, here, considering all the factors related to the enablement issue, it must be concluded that it would *not* require undue experimentation to make and use the subject matter defined in the claims. The conclusion is the claims are in compliance with 35 USC 112, first paragraph, as meeting the enablement requirement.

IV Definiteness

The Examiner has rejected Claims 4-5 and 9-12 under 35 U.S.C. 112, second paragraph, for being indefinite.

(i) The Examiner asserts the description "thioredoxin super family" in Claim 4 is ambiguous.

The Claim has been amended to remove this phrase, and now recites language (example sequences) the Examiner has indicated is supported by the specification.

(ii) The Examiner has asserted that since "thioredoxin superfamily" cannot be a single peptide, the description "wherein the thioredoxin superfamily is an isolated peptide having thioredoxin (TRX) activity" in Claim 9 is unclear.

This rejection should be overcome by the amendment as shown above.

(iii) The Examiner has asserted the description "thioredoxin activity" in Claim 9 is unclear.

The claim has been amended to recite the biological activity of "alleviating the symptoms of an inflammatory bowel disease". Therefore the claim should be considered definite.

(iv}The Examiner indicated that the description "peptide" in Claim 9 should be described "polypeptide".

The Claim has been amended in compliance with the Examiner's remarks.

(v) The Examiner asserts the description "the thioredoxin superfamily is in reduced form" in Claim 10 is improper since the thioredoxin polypeptide is already reduced.

In contrast to the Examiner's assertion, the polypeptide can be in both oxidized forms and reduced forms, as shown in the two references submitted herewith.

Hajime Nakamura et al.(2006 Seminars in Cancer Biology. 16:444-451), discloses that members of the thioredoxin family of proteins have a conserved catalytic site - Trp-Cys-Gly-Pro-Cys-Lys- that undergoes reversible oxidation to the cysteine-disulfide (Trx-S_2). The oxidized thioredoxine is reduced back to the cysteine-thiol form [Trx-$(SH)_2$] by the NADPH-dependent flavoprotein thioredoxin reductase (TR) (See Fig 1 and page 445, column 1, lines 1-4).

Garth Powis et al. (2000 Free Radical Biology and Medicine 29:312-322), discloses that reduced TRX with dithiol (-SH, -SH) reduces the protein disulfide and becomes oxidized to the disulfide (-S-S). Oxidized TRX is again reduced by thioredoxin reductase and NADPH. (see Page 312, Column 2, lines 2-14)

Therefore, TRX exists in both oxidized form and reduced form. Cysteine-thiol form (reduced form) can exert a catalytic activity. Oxidized TAX (cysteine-disulfide form) can be reduced in vivo. Accordingly, in the invention, both the oxidized form of TRX and the reduced form of TRX can be used. Claim 10 dependent on Claim 4 defines a mode of the invention using the reduced form.

In view of the above, the expression in Claim 10 is definite and the rejection should be withdrawn.

(vi) The Examiner asserts the description "the method...which is in the form of solution or suspension...." in Claim 11 is incorrect since the polypeptide is in the form of solution or suspension.

This rejection should be overcome by the amendment of Claim 11 to recite: "wherein a solution or a suspension comprising said polypeptide is used."

(vii) Claims 5 and 12 are rejected since they depend on claims which are indefinite.

The rejections of Claims 5 and 12 should be overcome by amending the parent claims as above.

CONCLUSION

In view of the above, it is submitted that the claims are in condition for allowance. Reconsideration and withdrawal of all outstanding rejections are respectfully requested. Allowance of the claims at an early date is solicited. If any points remain that can be resolved by telephone, the Examiner is invited to contact the undersigned at the below-given telephone number.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

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AMEND

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